Exhibit A

Exhibit A
For 10/590,848 1/5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Roberto PELLICCIARI

Serial No.: 10/471,549

Filed: 11 September 2003

FOR: STEROIDS AS AGONISTS FOR FXR

Group Art Unit: 1617

Examiner: B. BADIO

Atty. Dkt. No. 113847.123

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF ROBERTO PELLICCIARI PURSUANT TO 37 C.F.R. § 1.132

Madam:

I, Roberto Pellicciari, Ph.D., hereby declare that:

1. I received my Ph.D. in Chemistry from University of Rome and have spent more than twenty years involved in the fields of medicinal chemistry and bile acid research. I am currently a Professor of Medicinal Chemistry at the University of Perugia in Italy and an Adjunct Professor at the University of Maryland School of Medicine. I am also currently the President of the European Federation of Medicinal Chemistry. I have authored or co-authored more than 250 publications and am the named inventor on about 20 issued or pending patents.

3/5

2. I am the named inventor of U.S. Patent Application No. 10/471,549.

3. I have reviewed U.S. Patent Application No. 10/471,549, the Office Action dated November 3, 2005, and European Patent No. 312,867 to Frigerio et al., which was cited by the Examiner in the outstanding Office Action.

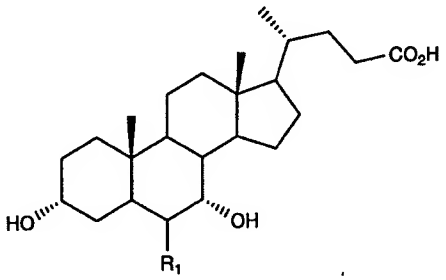
4. It is my understanding that in the Office Action of November 3, 2005, the Examiner has rejected claims 1, 2, 5-14 and 17-23 of the application as obvious in view of Frigerio *et al.* (EP 312,867). The Examiner has relied on Frigerio *et al.'s* description of 6-methyl substituted bile acid derivatives such as the 6-methyl derivative of chenodeoxycholic acid. Frigerio *et al.* is also relied on for teaching salts as well as conjugates thereof and for use of the compound in the treatment of biliary calculosis as well as pathological conditions in which the stimulation of biliary flow is required. As I understand it, the Examiner states that the 6-methyl derivative of chenodeoxycholic acid is an adjacent lower homolog of the claimed compound, and that my invention would have been obvious to one having ordinary skill in the art because he or she would expect the two compounds to have similar properties.

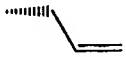
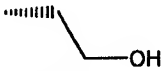
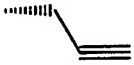
5. The Farsnesoid X Receptor (FXR), discovered in 1999, is a receptor for bile acid and chenodeoxycholic acid (CDCA). In an *in vitro* assay measuring the recruitment of the FXR co-activator SRC1 (Table 1), the 6-ethyl CDCA derivative of the present invention was unexpectedly and surprisingly more potent than the 6-methyl CDCA derivative of Frigerio *et al.*, as well as the natural ligand (CDCA) and other compounds shown in the table.

315

6. In the assay, the results of which are depicted in Table 1, the ability to bind FXR was tested. The SRC1 peptide is recruited in the presence of ligands to the receptor. The amount of binding was measured by Fluorescence Resonance Energy Transfer (FRET). The results show that the EC_{50} of the 6-ethyl CDCA derivative of the present invention was 0.098, whereas the EC_{50} of the 6-methyl CDCA of Frigerio *et al.* was 0.75.

Table 1. Binding Potency and Efficacy of Synthetic Bas to FXR



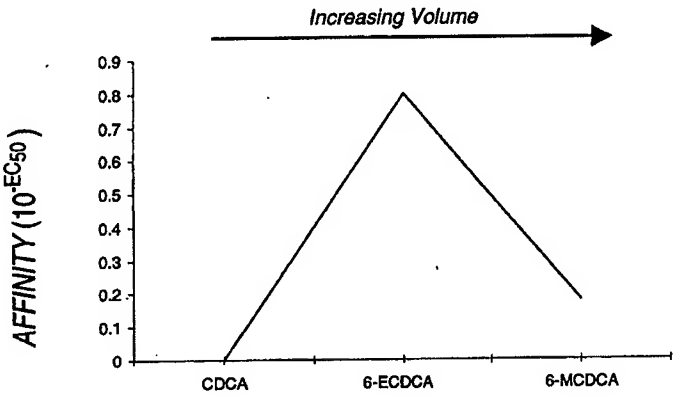
Comp.d	Trivial Name	R_1	EC_{50} (μM)	Efficacy (%)[a]
1	CDCA	H	8.66	100
2	6-Me-CDCA	Me	0.75	148
3	6-ECDCA	Et	0.098	144
4			0.48	170
5			61.15	68
6			0.54	105
7	HCA	α-OH	>30	9
8		α-OMe	14.73	113
9	6αFCDCA	α-F	15.11	99

[a]Relative recruitment of the SRC1 peptide to FXR where CDCA is 100%

415

7. The increased and superior affinity of the 6-ethyl CDCA derivative compared to CDCA and the 6-methyl CDCA is similarly depicted in Figure 1 below.

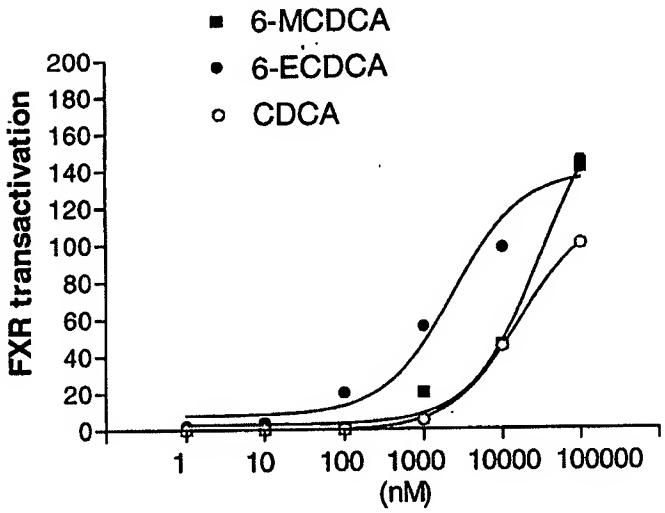
Figure 1



CDCA	2.19E-09
6-ECDCA	0.798
6-MCDCA	0.178

8. Furthermore, consistent with the FRET data, results obtained from *in vivo* transactivation assays carried out on HepG2 human liver cells (shown in Figure 2), demonstrate that the 6-ethyl CDCA derivative is significantly more potent than CDCA or the 6-methyl CDCA derivative.

Figure 2



5/5

9. The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Roberto Pellicciari, Ph.D.

Signed this 31st day of March, 2006.